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NEPHROLOGY FORUM

Immunoregulatory factors in the pathogenesis of IgA nephropathy

Principal discussant: STEVEN N. EMANCIPATOR

Case Western Reserve University School of Medicine, Cleveland, Ohio

Case presentations

Patient 1. A 17-year-old boy presented to his pediatrician 7 years ago complaining of bright red urine that turned brown over the 3 days prior to initial evaluation. At that time, he expressed concern that his recently initiated sexual activity was the cause of his problem. He otherwise was asymptomatic; a review of systems was entirely unremarkable. The family history was noncontributory; one grandparent, both parents, two sisters, and a brother were alive and well without known chronic disease. The three deceased grandparents all had died in their late sixties or early seventies of breast, colon, or lung cancer. No personal or family history of renal stones, urologic neoplasia, hematuria, deafness, urinary tract infections, or renal failure was elicited. Physical examination revealed a well-developed, well-nourished white male in no distress and without any discernible abnormality. Urinalysis revealed straw-colored urine with a specific gravity of 1.017, 1+ protein by dipstick, 15 to 20 erythrocytes/high-power field, and no casts. Urine culture was sterile. Because he had played a tough soccer game the previous week, hematuria was ascribed to strenuous exercise. He was reassured that occasional episodes of gross hematuria occur for many reasons in otherwise normal individuals, particularly after strenuous exercise. He was advised to return if he had red urine again.

During the ensuing 4 months, the patient had two additional episodes of gross hematuria, one documented by his pediatrician. Because of the patient's anxiety and the recurrent nature of the hematuria, he was referred for urologic evaluation. Several urinalyses revealed only 30 to 50 erythrocytes/high-power field or red cells too numerous to count. Cystoscopy, retrograde pyelography and cystography, and an intravenous pyelogram were normal.

On the day following the onset of yet another episode of bloody urine (now 22 months after the initial presentation), the patient was evaluated by a pediatric nephrologist, who documented macrohematuria; the patient's history and review of systems were remarkable only for the 4 episodes of gross hematuria. Close questioning revealed that in at least two instances, including the then-current episode, sore throat and a flu-like illness had accompanied the hematuria. Physical examination was remarkable only for pharyngitis, conjunctival injection, rhinitis, and an oral temperature of 38.7°C. Blood pressure was 142/90 mm Hg. Serum creatinine was 1.8 mg/dl; BUN, 48 mg/dl; albumin, 3.8 g/dl; glucose, 95 mg/dl; sodium, 142 mEq/liter; potassium, 3.7 mEq/liter; chloride, 98 mEq/liter; and bicarbonate, 26 mEq/liter. Urinalysis revealed a pH of 5.8, a specific gravity of 1.020, 2+ protein, 4+ blood, and numerous red blood cell casts and granular casts among erythrocytes too numerous to count. Urine protein excretion was 1.4 g/24 hr. One month later, the urine was clear, serum creatinine was 0.9 mg/dl; BUN, 12 mg/dl; and blood pressure, 118/75 mm Hg. Three more similar episodes occurred over the next 4 months; two occurred at the same time as an episode of pharyngitis and all were accompanied by mild acute renal insufficiency and transient hypertension.

The patient underwent a renal biopsy one day after the ninth episode of gross hematuria, 2 years after initial presentation. The biopsy revealed moderate mesangial hyperplasia and matrix hypertrophy, with segmental endocapillary proliferation in approximately 30% of glomeruli. No sclerotic glomeruli, crescents, synechiae, or foci of tuft necrosis were evident. No tubulointerstitial changes or vascular abnormalities were observed. Immunofluorescent examination revealed moderate to bright (2+ to 3+) granular mesangial deposits of IgA, C3, kappa and lambda light chains, and moderate (1+ to 2+) IgG in the same distribution. No significant IgM, fibrin, C1q, or C4 was evident. Ultrastructurally, mesangial hyperplasia and matrix hypertrophy were confirmed; numerous 500–1000 nm electron-dense deposits were found in the mesangial matrix, and rare similar deposits were apparent in the paramesangium. The capillary walls were normal, save for segmental irregular podocyte expansion. No other glomerular or tubular abnormality was observed ultrastructurally.

Seven and one-half years after initial presentation, this young man is active and healthy, with normal blood pressure and renal function. Episodes of gross hematuria occur 2 or 3 times annually, but renal function and blood pressure between episodes remain normal.

Patient 2. A 28-year-old man presented to his family physician because his prospective insurance company required evaluation of the 2+ proteinuria detected on his pre-insurance screening. He had been in his usual state of apparent good health and said that he felt fine. He was regularly physically active, and a review of systems was unremarkable. The patient was a new father of a baby girl. Both maternal grandparents and a maternal uncle had died of coronary artherosclerosis and myocardial infarction, and the paternal grandfather was being treated for prostate cancer. All other first- and second-degree relatives were healthy. There was no history of diabetes or renal disease in the family.

Physical examination revealed a healthy white male with a blood pressure of 154/95 mm Hg; no other abnormality was found. The fasting

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serum glucose was 88 mg/dl; BUN, 32 mg/dl; creatinine, 1.6 mg/dl; albumin, 3.8 g/dl; total protein, 7.5 g/dl; cholesterol, 325 mg/dl; and triglycerides, 182 mg/dl. Urinalysis revealed straw-colored fluid, a specific gravity of 1.012, a pH of 6.2, and 2+ to 3+ protein, but no ketones, blood, or bilirubin. Microscopic examination of the sediment revealed numerous granular casts but no red cell, white cell, or fatty casts. Followup over 6 months disclosed persistence of proteinuria, mild azotemia, and intermittent microscopic hematuria (15–50 red blood cells/high-power field). Persistent mild hypertension, documented during the first 2 months of followup, was treated effectively with hydrochlorothiazide and a diet without added salt. Urinary protein excretion ranged from 1.2 to 2.5 g/24 hr. Renal biopsy was performed.

Light microscopic evaluation of the biopsy specimen revealed glomeruli with equivocal to mild increases in mesangial cellularity, moderate expansion of mesangial matrix, and widely patent glomerular capillaries. The specimen contained no evidence of endocapillary proliferation, crescent formation, or tuft necrosis. Sclerosis, manifested by collapse of glomerular capillaries, was present in parts or all of 16% of the 164 glomeruli present in all levels of the histologic sections. The tubular interstitium contained rare foci of lymphohistiocytic infiltrates, which were associated with mild edema and degenerative changes in adjacent epithelium. No tubular atrophy or fibrosis was evident. The cortical arterioles manifested medial hypertrophy, hyperplasia, and hyalinosis, and these changes and intimal fibroelastosis also were evident in a lobular artery. Immunofluorescent microscopy revealed bright (3+) IgA, and moderate (2+) IgG, IgM, C3, and kappa and lambda chains, all in a granular mesangial pattern with some extension to capillaries. No C4, C1q, or fibrin deposition was noted. Ultrastructurally, expansion of mesangial matrix was accompanied by numerous electron-dense deposits from 300–800 nm in size, predominantly in the mesangium, but sometimes present in the paramesangium and glomerular capillary walls. In addition, there were rare foci of thickening of capillaries to approximately 500–600 nm, with lamellation and rarefaction of the lamina densa. Irregular segmental broadening of epithelial podocytes was evident.

Over 5 years, this patient has remained hypertensive; while receiving captopril, 25 mg twice daily, he maintains a blood pressure of 140/90 mm Hg. Proteinuria has remained in the 1.5 to 2.0 g/24 hr range, and microhematuria persists. Five and one-half years after initial evaluation, the serum creatinine has risen to 2.1 mg/dl.

Discussion

DR. STEVEN N. EMANCIPATOR (*Pathologist, University Hospitals of Cleveland, and Associate Professor of Pathology, Case Western Reserve University School of Medicine, Cleveland, Ohio*): Given the different pathologic and clinical features of these two patients, it is perhaps surprising that they share the same diagnosis: IgA nephropathy (IgAN). Diverse as these two examples are, they do not represent the clinical extremes of IgAN, namely, the acute nephritic syndrome with acute renal failure, and the nephrotic syndrome. Nor are diffuse, proliferative, necrotizing nephritis or minimal change by light microscopy—the pathologic poles of IgAN—illustrated by these two patients. In addition, whereas the second patient is likely to develop end-stage renal disease, the first patient is likely to maintain good renal function, at least for the next 20 years. Nevertheless, both patients are typical examples of IgAN, a syndrome defined by the predominant deposition of IgA within the glomerulus.

The first phase of investigation of IgAN consisted of a descriptive collation of clinical and/or pathologic features; several excellent recent reviews, including a prior Nephrology Forum, distill these original series, and reach similar conclusions about the disorder's attributes [1–7]. Nearly all patients with IgAN (95%) have hematuria sometime during the course of the disease. Its severity ranges from episodic macrohematuria (54% of patients), which often is superimposed on persistent microhematuria, to intermittent microhematuria. Proteinuria, the next most frequently observed sign (found in 85% of patients) generally is mild; about 50% of patients excrete less than 1 g of protein per day, but proteinuria in excess of 2 g/24 hr occurs commonly, and fully developed nephrosis is encountered in 5% to 10% of patients. Hypertension, acute renal failure, and chronic renal failure, observed to a variable degree and in various combinations, complete the clinical elements. By light microscopy, nearly two-thirds of patients have only mesangial abnormalities, manifest as some combination of cellular proliferation and matrix expansion. The severity and proportion of these features vary, not only from patient to patient, but from glomerulus to glomerulus within the same patient. Focal and segmental endocapillary proliferation, as in Patient 1, is associated with mesangial changes in another 20% of the patients, and another 14% have normal or minimally altered glomeruli. Diffuse endocapillary proliferation is present in only 5% of patients. Glomerular sclerosis, tuft necrosis, and/or synechiae accompany all patterns of glomerular injury, but with variable frequency and extent. By definition, IgA is the predominant immunoglobulin, but biopsy reveals deposits of IgG and/or IgM in most patients without systemic disease (75%). Alternative pathway and terminal complement components (C3, properdin, factor B, the C5b-9 membrane attack complex, and S regulatory protein) are nearly always present, but classical pathway components (C1, C4) are infrequent (12% of patients) [8–24]. Fibrin is not often deposited, except in crescents and foci of tuft necrosis. The immune deposits are nearly always in the mesangium, but they extend to the glomerular capillary wall in some cases (10%). Ultrastructurally evident electron-dense deposits in the capillary wall correlate with detection of capillary deposits by immunohistology. Increased mesangial matrix and hypercellularity seen by electron microscopy parallel the changes observed by light microscopy. Changes in the structural appearance of the capillary basement membrane itself are well documented but of unknown significance [25–27].

The second phase of investigation of IgAN charted the clinical course of large numbers of patients. Originally considered a benign disease with little or no potential for progression, we now know that IgAN results in end-stage renal disease in roughly one-third of patients. Again, the results of many large series, in which patients were followed for 10, 15, and even 20 years, are distilled in the several reviews I cited earlier [1–7]. Over the first 5 years, end-stage renal disease requiring dialysis or transplantation occurs in 1.0% to 1.5% of patients each year, and in 0.5% of patients in each subsequent year; an even higher incidence of chronic renal insufficiency is observed.

A significant milestone in our understanding of the natural history of IgAN was reached with the recognition that a subset of patients was at "high risk" for progression to total renal failure. The statistically significant features more frequent in the patients who ultimately develop chronic renal failure are: older age at onset, lack of macrohematuria, urinary protein excretion in excess of 1.5 to 2.0 g/24 hr, persistent hypertension, persistent azotemia, glomerulosclerosis, interstitial cortical fibrosis, circumferential crescents in more than 10% of glomeruli, extension of immune deposits from mesangium to glomerular capillaries, simultaneous abundant IgG or IgM deposits, and arteriolar hyalinosis. Particularly important is the absence of

macrohematuria, the presence of heavy proteinuria, persistent azotemia, and sclerosis of vessels and/or glomeruli in the biopsy; in several long-term series, these features consistently portend a poor outcome [6, 14, 28–34].

Syndromes that typically herald IgAN. The marked variety of clinical and pathologic features, as well as of long-term outcomes, evokes the question, how are individual patients with different attributes biologically related? Indeed, because IgAN as we currently diagnose it—that is, glomerulonephritis with predominance of the A class among glomerular immunoglobulin deposits in the absence of systemic disease—is so varied in clinical and pathologic expression, we must seriously consider whether IgA deposition is only an epiphenomenon. Beukhof and colleagues first proposed that a diagnosis of IgAN collects, under an inappropriate umbrella, otherwise unrelated diseases, each much more cohesive in clinical and pathologic detail and natural history [34]. I will discuss the issues of the propriety of the umbrella (IgA deposition as an epiphenomenon) and the relatedness of different manifestations of diseases later, but suffice it to say here that several investigators have categorized patients with IgAN into a few distinct groups, each quite cohesive in clinical features and prognosis [30–33]. Among these, two are most prevalent: those patients with episodic macrohematuria, often associated with acute renal insufficiency, as exemplified by Patient 1, and those with proteinuria associated with intermittent or persistent microhematuria, represented here by Patient 2. Henoch-Schönlein purpura, nephrotic syndrome, and chronic renal failure round out the clinical spectrum of glomerular disease with predominantly IgA deposits. The former two modes of presentation comprise respectively some 55% and 35% of patients with IgAN, while the latter three modes each represent roughly 3% to 5% of adults. In children, Henoch-Schönlein purpura accounts for 10% to 15% of patients with IgAN. The relationship of Henoch-Schönlein purpura to IgAN is well discussed elsewhere [35, 36] and does not bear repetition here. By definition, IgAN can only be diagnosed by renal biopsy. Hence, where asymptomatic microhematuria, alone or in association with low-grade proteinuria and normal renal function, is not an indication for biopsy, patients with these low-grade signs of disease do not contribute to the pool of IgAN patients. In locales where renal biopsy is performed in such individuals, approximately 60% of patients with this mode of clinical expression have predominantly IgA and C3 deposits, and therefore IgAN [37, 38]. In most nations, signs more overt than microhematuria are required for biopsy, and patients therefore usually fall into two basic groups: those with episodic macrohematuria and those with microhematuria and proteinuria.

I believe that these two prominent modes of disease expression, that is, episodic macrohematuria and asymptomatic microhematuria with proteinuria, are indeed related pathogenetically, pathophysiologically, and immunologically, and that the diagnostic term IgAN is not an inappropriate umbrella. Patients who present with episodic macrohematuria tend to be younger, with bouts of hematuria closely associated in time with pharyngitis, coryza, or other "flu-like illness." Although one-half of all patients with IgAN give a history of viral illness associated with disease onset, patients with episodic macrohematuria comprise most of the patients with pharyngitis occurring concomitantly with nephritis. Conversely, few IgAN patients lacking a history of a "viral syndrome" associated with nephritis have macroscopic hematuria. Infectious episodes, then, seem to precipitate episodic macrohematuria. A more subtle element, as yet undefined, appears to underlie the microhematuria/proteinuria mode of disease expression.

I believe that in each of these modes of clinical presentation, distinct defects in the regulation of immune responses, particularly of mucosa-associated lymphocytes, might well explain the pathogenesis of IgAN. Indeed, aberrations in immune function and lymphocyte markers have been recognized in IgAN, although such studies have not categorized patients into the aforementioned clinical subgroups. Review of these issues of disordered immune regulation permits development of a new, working hypothesis on IgA nephritogenesis. In this Forum, I will define IgAN as an immune-complex glomerulonephritis with IgA antibody specific for unknown foreign antigen(s); this perspective is based on reasons presented elsewhere [5, 7]. Others have proposed that IgA with autoimmune specificity, particulary IgA rheumatoid factor or IgA aggregated for non-immune reasons, are sources of IgA deposits in glomeruli. Some investigators have contended that the IgA in glomerular deposits is in fact the antigen component of immune complexes with anti-IgA antibodies. In all these alternate hypotheses, IgA must be synthesized. However, in all cases, IgA synthesis is likely subject to the same isotype-specific regulation as physiologic IgA antibody synthesis. Hence, the influences on regulation of IgA production I will discuss presently apply to nephritogenesis under these alternate hypotheses as well, although perhaps with modification.

Special features of regulation of mucosal immune responses. IgA has a special relationship to mucosae and to mucosa-associated lymphoid tissue [39, 40]. Some plasmacytes within the bone marrow, and also in spleen and lymph nodes remote from mucosae, contribute to the total-body IgA pool. But IgA production by these latter tissues is far overshadowed by cells derived from mucosa-associated B cells. These B-cell-derived cells arise, differentiate, and expand in mucosal-associated sites and, after circulating systemically, travel to distant as well as adjacent mucosal sites. These cells reside in the lamina propria as differentiated plasma cells. Considerable evidence indicates that distinct regulatory T-cells also develop in mucosae; these cells are thought to influence not only the B-cell progeny of mucosa, but also B-cell progeny in marrow and lymphoid organs distant from mucosae. Together, these regulatory T-cells and B-cells form a "mucosa-marrow axis" [41–43]. The amount and class distribution of antibody produced by plasmacytes, be they residents of marrow or mucosae, are subject to the regulatory influence of T-cells. One such mechanism of regulation, subtended by mucosal T-cell subsets, is the phenomenon of "oral tolerance" [44, 45].

I will elaborate here on this notion of "oral tolerance." Typically, exposure to antigen via a mucosal route initially leads to sensitization to the antigen. This exposure elicits IgM, then IgG, and finally IgA antibodies, as well as cytotoxic and factor-producing antigen-specific T-cells. However, protracted antigenic exposure at a mucosal site results in elimination of IgM and IgG antibody production, and in a loss of effector T-cells [44–47]. Occasionally, loss of the humoral response includes loss of IgA, but more frequently the loss is selective, increasing IgA production, while diminishing IgM and IgG

synthesis. Genesis and maintenance of this state, termed "mucosal tolerance" or "oral tolerance" (owing to the predominance of experiments that utilize the gut as the site of antigenic exposure), comprise an active process governed by several regulatory T-cell subsets [48–51]. Although human data are relatively limited, this mechanism appears to apply to humans as well as to mice [52, 53]. Evidence that the process is active derives from experiments in which tolerance was adoptively transferred by T-cells and was "broken" by drugs that are toxic for T-cells or that interfere with antigen presentation to regulatory T-cells [48–51]. Prominent among these T-cell subsets are "switch" cells, which promote rearrangement of immunoglobulin heavy chain genes in B-cells [54, 55]. After rearrangement, the variable portion of the molecule, with its intact binding site for antigen, is spliced to the heavy chain constant regions farther out along the heavy-chain gene sequence. The two subclasses of IgA, flanking IgE, another "mucosa-associated" class of immunoglobulin, lie at the end of the human gene sequence. By promoting expression of "late" heavy-chain genes, switch T-cells can select for IgA and IgE expression at the expense of IgM and IgG. Helper T-cells specific for IgA-committed B-cells also have been described; again these helper T-cells would select for IgA expression [56]. Cells that are phenotypically and functionally suppressor cells also can augment antibody responses; such "contrasuppressor cells" also can select for IgA by suppressing the function of IgA-specific suppressor T-cell subsets [57–59]. T-cells selective for IgA are enriched in mucosa-associated T cells, when compared with spleen-associated T-cells.

In addition to these cell-based mechanisms that require cell-to-cell contact, selection for IgA can be favored by elaboration of soluble materials from T-cells. For example, populations of T-cells can promote a switch to IgA commitment and differentiation of B-cells from remote sites by secreting IL-4 and IL-5 [60–63]. Factors that bind to a particular class of antibody are secreted by T-cells and can directly influence B-cells as well as inducer T-cells and effector T-cells involved in antisuppression/contrasuppression [64]. Different isotype-specific binding factors can, of course, suppress B-cell function committed to production of different antibody classes [65–67].

Potential influence of mucosal immunoregulation in IgA nephritogenesis. The complicated and still-evolving area of mucosal immunoregulation, although somewhat nebulous, offers insights into the pathogenesis of IgA nephropathy. As expected, regulation of the IgA immune response can elicit either noxious or innocuous antibody responses. As I stated earlier, protracted mucosal exposure to antigen normally results, via a poorly understood and concatenated mechanism, in an immune response that almost exclusively produces IgA. In an environment where large amounts of foreign material daily contacts the body, IgA is teleologically an ideal defense mechanism. Phylogenetically, IgA probably contributed to the survival of the species [68]. It is actively transported by a specific receptor on the epithelium lining mucosal surfaces, and thus IgA exists in high concentration at the interface between the body and foreign materials of the outside world [39, 40, 68]. The potent agglutinating ability of IgA can inhibit attachment and motility of organisms. Also, IgA can mediate antibody-dependent, cell-mediated cytotoxic and phagocytic responses by interacting with specific receptors present on phagocytes [69, 70]. Moreover, binding of IgA to macromolecules within the mucosal lumen inhibits penetration of those macromolecules into the lamina propria and, therefore, the body proper [71]. Yet IgA seems to be a poor initiator, and perhaps an inhibitor of, complement fixation [72]. This seeming defect paradoxically is likely an advantage in the mucosal environment; were complement fixed, the epithelium might be damaged, the integrity of the epithelial barrier might be breached, and foreign material could penetrate freely into the host [73]. Chemotactic recruitment of phagocytes to the area of complement fixation might create a state of chronic inflammation and amplify such injury.

If mucosal tolerance fails, the aforementioned benefits of an IgA response would be lost, and IgG synthesis rather than IgA would be dominant. Substitution of monomeric IgG at the expense of dimeric IgA simultaneously would result in less agglutination, and, because IgG is not actively secreted, the concentration of antibody in the mucosal lumen would be lower, thus favoring entry of antigen. Such IgG antibody, and antigen-specific cytotoxic and factor-producing T-cells, which encounter antigen on the surface of epithelium, might produce epithelial injury. This scenario would further favor penetration of foreign materials. Hence, a failure of "mucosal tolerance" can be predicted to increase the load of antigen that gains access to the lamina propria, and ultimately to the circulation.

What of the material that does gain access to the circulation? In the presence of a normal IgA immune response, IgA antibody, concentrated in the mucosal lamina propria, generates immune complexes (IgA complexed with viral or bacterial pathogens, or food or environmental antigens) subject to clearance by the mononuclear phagocyte system. The relatively small amount of complexes that escape such clearance are then subject to deposition in glomeruli. The resultant deposits, with almost exclusively nonphlogistic IgA, are unlikely to evoke glomerular injury in the interval before they are naturally cleared, however.

Failure of mucosal tolerance would favor a mixed-isotype immune response, with persistent IgG and IgM antibodies. In addition to increased penetration of the mucosa by antigen (expected for the reasons I just presented), the complexes that do gain access to the mesangium would be more likely to be noxious because of their IgG and IgM content, which promotes complement deposition (at least in experimental mice) [74]. Mice parenterally challenged with a specific antigen to which a mucosal immune response has been evoked accumulate glomerular deposits of IgG and/or IgM and complement, and these animals develop hematuria if challenged prior to the onset of "oral tolerance." The mice develop only IgA and antigen deposits, however, if challenged in the setting of a "pure" IgA immune response after achievement of "oral tolerance." In parallel experiments, in which immune complexes were prepared in vitro and injected into mice, the admixture of IgG (capable of fixing complement via the classical pathway) into IgA-predominant complexes promoted glomerular complement deposits and proved nephritogenic. In contrast, administration of IgA-predominant complexes containing a structurally similar IgG (incapable of fixing complement) did not produce hematuria, proteinuria, azotemia, or glomerular complement deposition [74].

The concept that admixed IgG and IgM produce glomerular disease appears to apply to patients as well as to experimental

animals. Patients with IgAN who are biopsied because of overt renal manifestations typically also have IgG and/or IgM deposits. In contrast, immunohistologic study of asymptomatic patients, many from autopsies in patients with hepatobiliary disease, often reveals mesangial IgA deposits, but without concomitant IgG, IgM, or C3 deposits [75–79]. Exacerbation of IgAN is associated with acute elevations in the IgG content of circulating immune complexes and with increased differentiation of IgG-producing cells in the blood [80]. Finally, the heavy admixture of IgG and/or IgM, at least in some studies, is observed in more severely affected patients, who have a greater propensity for progression of their renal disease [81–83].

The prediction that failure of "oral tolerance" can give rise to glomerular deposits containing IgA with IgG and/or IgM, and promote a greater propensity for injury, is confirmed by studies in mice. Gesualdo et al gave two propitiously timed doses of estradiol or cyclophosphamide to mice that had been orally immunized continuously for 14 weeks with bovine gamma globulin [84]. Both these drugs interfere, by different mechanisms, with the genesis of "oral tolerance" [48–51]. Compared with identically immunized mice given saline as a control, mice given estradiol, cyclophosphamide, or both developed identical serum antibody titers to the oral immunogen by 4 weeks; IgA antibody in serum continued to rise and was identical in all groups throughout the 14-week experiment. As anticipated, "oral tolerance" began at 6 weeks, and thus the group given saline showed a decline of IgG and IgM antibodies. Further, titers of these antibodies were indistinguishable from nonimmune age-matched control mice by 10 weeks. In contrast, the IgG antibody titers in the serum of mice given estradiol, cyclophosphamide, or both continued to rise until 8 weeks, and these titers were maintained at a high level until the mice were sacrificed at 14 weeks. As would be expected from the immune response, in approximately 80% of the mice given estradiol, cyclophosphamide, or both, mesangial deposits contained appreciable amounts of IgG and C3, which accompanied the predominant IgA. As with our earlier experiments [74], the mice with a combination of IgG and C3 with IgA in the deposits had microhematuria and mild proteinuria. Mice given saline had comparable amounts of IgA, but lacked IgG or C3 deposits, hematuria, or proteinuria [74, 85].

In light of this new information on mucosal immunoregulation, we can envision generation of abundant immune complexes containing some IgG and IgM, but mostly IgA antibody, via two routes. First, large amounts of antigen(s) might penetrate the mucosae prior to the development of "oral tolerance." Second, the host might fail to establish isotype switching and "oral tolerance" fully.

In the former situation, increased mucosal penetrability to antigen, and/or an overexuberant and particularly intense, mixed isotype humoral immune response to the antigen, would promote rapid accumulation of abundant immune complexes within the body proper. Enhanced mucosal penetrability per se is not well established in patients with IgAN, although some investigators have implicated increased uptake of various common dietary and infectious antigens in IgAN patients [7, 86]. Infection with a pathogen, however, presupposes penetration by the organism. In mucosal infections in general, antigen penetration does not lead to IgAN; that is, most patients afflicted with influenza, for example, do not develop clinically evident renal disease. However, with the same load of antigen, more robust and rapid antibody responses might induce nephritis. This situation can be produced in the experimental mouse model [87]. Reinfection of immune mice with a parainfluenza virus does not elicit nephritis. However, parenteral challenge with a standard infectious dose of virus, or respiratory challenge with a hundredfold more virus than the usual infectious dose, is nephritogenic in immune mice, if the virus is infectious rather than inactivated [87]. These mice developed macrohematuria and mild acute azotemia reminiscent of the hematuria accompanying pharyngitis in patients with IgAN. Abnormally low levels of antibody after an infection (low immunologic memory) would predispose to reinfection with the same or a serologically related strain of virus, and therefore to an abnormally high mucosal penetrability by antigen, relative to normal immune subjects. This situation is analogous to mucosal challenge with a hundredfold more virus in a normally immune host. When coupled with abnormally accelerated antibody synthesis after antigen challenge, lower memory antibody levels would prove an ideal milieu in which infections could at once penetrate mucosae and generate a heavy immune complex load.

Increased specific IgA antibody was observed in the serum of many IgAN patients shortly after mucosal challenge with polio vaccine and other viral antigens in three independent series [88–90]. In addition, Waldo and Cochran demonstrated accentuated decreases in in-vitro production of specific IgA antibody later on, in the presence of increases in total IgA and specific IgG antibody [88]. Hence, the prediction of hyperfunction both of helper T-cells (during antigen challenge) and suppressor T-cells (during antigen-free intervals) as an ideal milieu for IgA nephritogenesis is documented in at least some patients with IgAN.

It is conceivable that in the subgroup of IgAN patients with episodic macrohematuria associated with acute "viral" infection, bouts of nephritis result from such a phasic hyperfunction of helper and suppressor T-cells. It is well to note in this context that the antigen need not be, and likely is not, a single chemical or biologic entity, but might be several distinct elements. Serologic variants of organisms would be, at least to the extent of the new antigenic determinants, *primary* antigens. Moreover, this proposed mechanism is not incompatible with hypotheses that propose IgA as a rheumatoid factor or an antigen component of immune complexes, because the IgA production instigated by a viral infection could participate in complexes via these mechanisms.

A second route to increased generation of mixed-isotype immune complexes with predominant IgA derives directly from the body's failure to fully establish isotype switching and "oral tolerance." In this situation, a commensal organism or a dietary or environmental macromolecule would evoke "mucosal tolerance" in normal individuals but would produce a mixed-isotype response, with its attendant promotion of antigen penetrance and mixed-isotype complexes, in patients with failure of "oral tolerance." This aspect needs to be explored in patients with IgAN, but a mixed-isotype response to persistent mucosal antigens with failure of mucosal tolerance might account for the subgroup of patients with microhematuria and proteinuria. Abnormalities in IL-4 levels in IgAN patients' sera and culture supernatants might provide the first clues in this area.

Alterations in immunoregulation in patients with IgAN. Further evidence of defective immunoregulation in IgAN lies in the variety of abnormalities in lymphocyte function described in patients with IgAN and their relatives, compared with patients with other forms of glomerulonephritis and normal individuals. The details are extensive and beyond the scope of this Forum; the reader is referred to several comprehensive reviews and key papers [5–7, 91–107]. To summarize briefly, approximately one-half the patients with IgAN have increased serum levels of IgA. Peripheral blood B-cells from many patients with IgAN show abnormal spontaneous IgA secretion and, more consistently, increased IgA production after mitogen stimulation. Several investigators also reported increased fractions of peripheral blood B-cells expressing surface IgA, and production of IgA by peripheral blood B-cells in the presence of mitogen-activated T-cells capable of suppressing IgA synthesis by B-cells from non-IgAN patients [93–97]. Some of these observations have not been confirmed, however [98–100]. Investigators also have described increased specific IgA antibody responses in serum to a variety of antigens [88–90]. Oral administration of polio vaccine to IgAN patients, first-degree relatives, and controls revealed upregulated serum IgA antibody in patients and relatives and, paradoxically, showed diminished production of specific IgA in lymphocyte culture supernatant from IgAN patients [88]. These findings suggested IgA-specific suppression [88]. There also appears to be selective expression of some IgG subclasses in IgAN patients relative to controls, with reciprocal differences observed in serum and glomerular immune deposits [101, 102]; these observations suggest altered regulation of immunoglobulin isotype expression in patients with IgAN. In addition, intriguing evidence implies that genetic differences in heavy-chain switch regions, where the variable portion of antibody heavy chains is spliced to the constant portion of a specific antibody class, are more frequently encountered in patients with IgAN than in normal individuals in the general population [103, 104].

Peripheral blood T-cells from IgAN patients, and culture supernatants from these T-cells, are less effective at suppressing IgA secretion by mitogen-stimulated B-cells than are identical preparations from normal controls [94–97, 105]. The IgA-specific suppressor T-cell activity in IgAN patients correlated inversely with serum IgA levels and the percentage of B-cells in blood that express IgA on their cytoplasmic membranes [94–97, 105–107]. Patients with IgAN also manifest increased function of T-cells, which promote IgA secretion in vitro, and increased percentages of T-cells with surface receptors for IgA in the circulation [94–100, 105–107]. As with the B-cell data, not all investigators agree on all details.

Particular emphasis is needed in two areas to refine our understanding of immunoregulation in IgAN: the difference between regulatory status in exacerbation versus remission, and the distinction between mucosally derived lymphocytes versus lymphocytes derived from "peripheral" lymphoid organs, that is, non-mucosa-related lymphocytes. Egido and colleagues did observe increased polymeric IgA-producing blood lymphocytes in patients during exacerbation, an observation suggesting mucosal-specific alterations [94, 107, 108], but several other groups have not [95–97]. Efforts at employing the tonsil as a source of mucosal lymphocytes have yielded findings essentially similar to those reported with peripheral blood lymphocytes [108, 109], but because the tonsils are a "crossroads" both for systemic and mucosal lymphocytes, rather than a true mucosa-associated lymphoid tissue, the results with tonsils are neither surprising nor specific for a particular lymphoid component. Studies of lymphocyte function in IgAN patients in exacerbation versus remission are limited, but Schena et al reported that in exacerbation of IgAN, peripheral blood lymphocytes in vitro differentiated to IgG- as well as to IgA-producing cells [80]. Moreover, the IgG content of immune-complex-like material and serum IgG levels were elevated in exacerbation compared with remission. Such changes were encountered more frequently in patients with more severe cases of IgAN, but it is difficult to distinguish the immunologic effects of azotemia from fundamental differences in immune status that antedated renal disease.

Most recently, Gesualdo and coworkers observed differences in interleukin (IL)-4 production by resting and mitogen-stimulated peripheral blood lymphocytes and in serum IL-4 levels in patients with IgAN compared with age- and gender-matched normal volunteers (Gesualdo L, personal communication). Reminiscent of the results Waldo and Cochran obtained with specific IgA immune responses to oral polio vaccine [88], and of some results in lymphocyte studies [94, 105, 107, 109], production of IL-4 by peripheral blood lymphocytes from patients with IgAN was double that by lymphocytes from normal individuals, whereas the serum IL-4 content in the patients was only 60% of that in controls. These responses suggest either increased catabolism or increased receptor-mediated uptake of IL-4 in patients with clinically evident IgAN. More extensive work obviously is required to fully characterize the immunoregulatory state in specific groups of patients with IgAN. All results to date suggest some, perhaps multiple, immunoregulatory defects in patients with IgAN; the nature of these defects can differ among different subpopulations of patients.

Genetic aspects of IgAN. Although agents such as cyclophosphamide and estradiol can interfere with the establishment of "mucosal tolerance" in mice [84], postnatal failure of mucosal tolerance is not generally recognized as a cause of defects in mucosal immunoregulation. On the other hand, significant evidence has been adduced that heritable factors influence the risk of developing IgAN. Familial clustering [110–117] and racial predilection [118] or sparing [119] are well recognized in association with IgAN. Numerous reports have associated the severity of, or risk for, IgAN and major histocompatibility gene loci, notably in the DR/DQ immune-associated (Ia) regions [5, 6, 120] and the class-III genes for complement component 4 [121, 122]. Other genes or gene segments implicated are the switch regions of immunoglobulin heavy-chain genes [103, 104] and the genes for complement component 3 [121, 122]. This abundance of data is tantalizing but difficult to evaluate as yet; at present, the only confirmed and uncontested data apply to the complement phenotypes, as reviewed elsewhere [123]. Nonetheless, genetic studies support an immunoregulatory defect in at least some patients with IgAN. Such genetic influence might underlie abnormal lymphocyte function in patients with IgAN as well as their first-degree relatives, as I already noted [5–7, 88–100, 105–109, 124].

The mechanism of injury in IgAN. The mesangium contributes appreciably to glomerular pathophysiology [125], including the genesis of proteinuria [126]. Immune complexes, the mem-

brane attack complex of complement, and cytokines from inflammatory cells promote functional and morphologic changes in the mesangium; these changes include calcium mobilization and mesangial contraction. They also include the release of multiple substances including vasoactive prostaglandins, platelet activating factor, proteolytic enzymes, reactive oxygen intermediates, and cytokines such as interleukin-1 and tumor necrosis factor [127–140]. Mesangial cells also can proliferate upon insertion of membrane attack complex into cell membranes [133]. Mesangial cells synthesize platelet-derived growth factor, an autocrine mitogen [141, 142], and this synthesis can be augmented after stimuli such as growth factors, eicosanoids and cytokines, in turn stimulated by immune complexes and complement. The precise interplay among these mechanisms and metabolites remains unclear, and we are only beginning to understand the influence of IgA on their modulation. My own belief is that the basic principles of mesangial structure, function, and interaction with immune reactants underlie glomerular function in IgAN and other forms of glomerulonephritis equally.

IgA deposition as an epiphenomenon. Save for macrohematuria, the factors governing long-term prognosis in IgAN are very similar to those in membranous nephropathy [143], and possibly are generic for glomerular disease in general. Also, histologically and clinically matched cases of mesangial proliferative glomerulonephritis, with and without dominant IgA, have similar long-term courses in Japan and New Mexico [144, 145]. A minority of patients with IgAN (25%) have IgA as the only immunoglobulin deposited, whereas many asymptomatic patients with liver disease have abundant mesangial IgA deposits but have no overt renal disease [75–79]. As noted before, in experimental mice with IgAN induced by oral immunization or injection of immune complexes, concurrent deposits of IgG and IgM are important elements, apparently because they promote glomerular C3 deposits, which are potent at nephritogenesis [74]. In another experimental system induced by injection of immune complexes, IgG/IgM deposits are unimportant, but the antigen structure is critical for nephritogenesis [146]. These observations collectively suggest that IgA per se is neither noxious to the glomerulus nor intrinsically pathophysiologically important.

An alternative view holds that the IgA component of glomerular immune deposits in IgAN is a functionally important ingredient in immune deposition or pathogenesis. Introduction of IgA into an immune lattice, even as small percentages of the total immunoglobulin, profoundly affects the complement-activating potential of that lattice; it appears that IgA somehow *inhibits* IgG- and/or IgM-mediated complement fixation [72]. Therefore, IgA-rich immune complexes likely have a complement:immunoglobulin ratio quite distinct from similar complexes lacking IgA. This ratio could significantly influence the propensity of immune complexes for depositing in the kidneys. In primates, IgA immune complexes are cleared from the circulation more slowly than are similar IgG immune complexes with the same molecular weight and charge, presumably because of the less efficient operation of the erythrocyte complement receptor (CR1) clearance mechanism on the relatively C3b-poor IgA complexes [147]. Hence, IgA immune complexes are deposited more widely than are similar IgG immune complexes; glomerular deposits of IgA immune complexes are threefold that of IgG immune complexes. The existence of defects in clearance of IgA immune complexes in patients with IgAN versus normal volunteers remains controversial [148, 149]. Even in the absence of defective clearance, however, a predilection for IgA incorporation into immune complexes would favor glomerular deposition of immune complexes relative to patients with less propensity for IgA synthesis; formation of complexes with the same total amount of specific antibody, but a higher percentage of IgA, would favor less complement fixation, impaired clearance, and more generalized immune deposition.

Another means by which IgA might play a pathogenetic role is by virtue of its binding to fibronectin (Woodroffe A, Schlondorff D, independent personal communications) [150]. Fibronectin is an abundant constituent of the extracellular matrix of the glomerular mesangium. According to one hypothesis, IgA immune complexes or perhaps IgA alone would preferentially accumulate in the mesangium by virtue of the binding of IgA to the fibronectin component of the glomerulus. If IgG and/or IgM and C3 were associated with these immune complexes, they likewise would deposit and initiate nephritis. Some investigators have posited that IgA immune complexes might deposit first, followed by accumulation of IgG and/or IgM with specificity either for an antigen component, or for the IgA, within the IgA immune complexes. In either event, the affinity of IgA for fibronectin could serve as a means of "targeting" immune complexes to the mesangium. In addition, IgA immune complexes could influence mesangial cell metabolism by interfering with cellular interactions with fibronectin. Other, analogous interactions of IgA and IgA immune complexes with the glomerulus are possible.

Finally, the relationship between IgA and IgG and complement components of glomerular deposits might not be at the level of initiation of injury but rather at the level of amplification of injury. Hernando et al recently reported that immune complexes prepared in vitro with only IgA antibody and similar immune complexes prepared with only IgG both elicited superoxide anion and tumor necrosis factor release from mesangial cells in culture [140]. Although both isotypes elicited comparable responses, the addition of fresh serum as a source of complement augmented the response to (complement-fixing) IgG-containing immune complexes, but the fresh serum did not influence the response to IgA immune complexes [140]. If these in-vitro data can be extrapolated to in-vivo conditions, they provide several explanations. First, adding sufficient IgG (or perhaps IgM) to a predominantly IgA immune lattice would favor some complement fixation, albeit much less than would obtain were all the antibody of the IgG or IgM class. Complement fixation then could augment mesangial cell responses, presumably including contraction, and production of eicosanoids, IL-1, superoxide, and tumor necrosis factor. On the other hand, if the antigen itself could promote complement fixation, concurrent IgG or IgM deposits would be unnecessary for the synergistic effects of complement. Moreover, if the antigen structure resulted in a high density of IgA antibody, the local concentration of IgA alone might be high enough to elicit maximal mesangial cell responses, and augmentation by complement would be unnecessary.

Conclusion. The subject of IgAN is complicated and provocative. Glomeruli likely respond to IgA deposits as they do to

any immune stimulus, perhaps with minor variations. The IgA itself, while apparently not directly noxious, seems to constitute a physiologically important element and to be a marker associating mucosal antigenic exposure with disease. Specifically, IgA may impair complement-mediated clearance of immune complexes from the circulation and/or promote mesangial deposition of complexes by binding to endogenous glomerular components, such as fibronectin, perhaps on a lectin-like basis.

I believe that the primary defect in IgAN lies within the regulation of the immune response. Either of two defects might exist. Overly wide swings in antibody production, due to hyperfunction both of suppressor T-cells (during antigen-free intervals) and helper T-cells (during periods of antigen exposure, which incite disease exacerbations), appear to be one defect. The other potential defect is inadequate suppression of "systemic" IgG and/or IgM antibody responses to persistent mucosal exposure to environmental or dietary antigens. These specific defects likely give rise, respectively, to the major syndromes of IgAN: episodic macrohematuria and persistent proteinuria and hematuria. I hope that tangible, convincing data soon will emerge from the circumstantial evidence now framing the perspective of IgAN as a primary disorder of the immune system. Recognition of specific immune defects might also permit noninvasive specific diagnosis of IgAN and perhaps will allow specific therapy in the future.

Questions and answers

DR. JOHN T. HARRINGTON (*Chief of Medicine, Newton-Wellesley Hospital, Newton, Massachusetts*): If your hypothesis is correct, that IgA deposition depends on altered production of IgA and IgG, which in turn depend on IL-4 function, one would speculate that IL-4 production would be stimulated when IgA nephropathy flared and that cellular IL-4 production would be normal when patients were in remission. Have such studies been performed?

DR. EMANCIPATOR: The distinction between active disease and disease in remission, particularly in the episodic macrohematuria group, is absolutely critical. Part of the reason that we've had trouble in understanding the lymphocyte function data is because we haven't made the distinction between patients in remission versus those in exacerbation. Nor have we made the distinction between patients with episodic macrohematuria versus those with persistent proteinuria and microhematuria. In answer to your question, the patients with active episodic macrohematuria produced 300 to 400 pg/ml IL-4, whereas patients in remission synthesized 35.3 ± 4.8 pg/ml. In contrast, normal volunteers and patients with glomerulonephritis without IgA immune deposits produced only 12.9 ± 3.9 and 11.3 ± 4.5 pg/ml, respectively. I think these are critical controls regarding the IL-4 data.

DR. HARRINGTON: Do you have control data of another sort, that is, data from active patients with Henoch-Schönlein purpura?

DR. EMANCIPATOR: I think that Henoch-Schönlein purpura nephritis should also be stratified according to active versus remission stages. I suspect that lymphocyte function studies will reveal results similar to IgAN with episodic macrohematuria.

DR. JAMES STROM (*Chief, Division of Nephrology, St. Elizabeth's Hospital, Brighton, Massachusetts*): If I understood you correctly, you postulated a defect in mucosal IgA processing in these patients, which leads to overstimulation of IgG, which in turn causes nephritis in the presence of complement. Why, then, do these patients demonstrate prominent IgA deposition?

DR. EMANCIPATOR: I didn't mean to imply that I believe there is a defect in IgA processing in the gut of patients with IgA nephropathy. I am not aware of any evidence, nor do I have the bias, that there's a defect in IgA processing in these patients versus normal people. In fact, many patients with IgA nephropathy have overabundant IgA production, and I'm not aware of any difference in secretory IgA levels in that population. Presumably if serum IgA is increased, then IgA secretion is increased as well. The problem, persistence of IgG production, reflects a defect not in IgA processing but rather in T-cells turning off IgG production. On the other hand, your question does bring up the possibility that if in fact there were a defect in secretory component-mediated transport of IgA or in local immunity, a patient would have serum IgA but not mucosal IgA. Some individuals do have such a condition. These people would have a higher risk of mucosal infection, but normal serum antibody, potentially rich in IgA, capable of generating immune complexes with the antigen, which enters the body consequent to deficient local immunity. The immune complexes in turn could localize within the kidney.

DR. MICHAEL MADAIO (*Division of Nephrology, New England Medical Center, Boston, Massachusetts*): I know you've spent the last hour discussing this, but could you summarize your working hypothesis for the mechanism of immune deposit formation in IgA nephropathy?

DR. EMANCIPATOR: My working hypothesis is that the antigen penetrates and combines with the antibody in the lamina propria of the airway or intestine, gains acccess to the circulation, and deposits as such. Perhaps some immune complexes also form within the mucosal lumen and are transported, but it's fairly well established that immune complexes are less likely than the free antigen to penetrate through the mucosal barrier.

The idea of in-situ formation, although not emphasized in the context of mesangial deposits, merits further consideration. In-situ mechanisms traditionally have been emphasized in association with epimembranous deposits, largely because it is difficult to elicit such deposits by injecting pre-formed immune complexes, whereas mesangial and subendothelial deposits are readily formed. Mesangial deposits may form via in-situ mechanisms principally, or in concert with deposition of circulating immune complexes. I already mentioned fibronectin-IgA binding. Antigens could bind to mesangium, and then IgA in turn could bind to antigen. In fact, Amore has demonstrated just such a mechanism with gliadin as antigen [151].

DR. MADAIO: Even during periods of clinical remission, patients with IgA nephropathy typically have glomerular deposits of IgA. What factors precipitate clinical episodes of nephritis? Is it purely a quantitative phenomenon? Or is it related to a qualitative factor such as either deposition of complement-fixing antibodies or an isotype switch to IgG?

DR. EMANCIPATOR: I believe the factor is largely qualitative. In some cases, the IgA persists without clinically evident nephritis. A few reports suggest that the IgG and C3 are less abundant during remission [3, 18, 29, 31, 83], but this evidence is scanty at best. Patients with cirrhosis of the liver or hepato-

biliary disease have been used as a model; they have abundant IgA deposits, yet the vast majority of these patients have no clinical manifestations of renal disease. We had speculated in *Clinical Nephrology* a couple of years ago that it's the IgG production that distinguishes exacerbation from remission [5]. My belief has now been substantiated in IgAN patients by Schena [80].

DR. HARRINGTON: You mentioned that patients with the episodic gross hematuria mode of presentation of IgAN, particularly, develop macrohematuria within hours of a clinical viral illness such as pharyngitis. You have proposed that subsequent to viral infection, antibody specific for viral antigens is produced and combines with the antigens, generating immune complexes that deposit in the kidney. Can one produce enough antibody within the first few hours of a viral pharyngitis to account for all the changes in exacerbation?

DR. EMANCIPATOR: The rate of the immune response depends on whether the responding antigen-committed cells are lymphocytes, plasma blasts, or plasmacytes, and where in the body these cells reside relative to antigen entry. Viral infections, and less so bacterial infections, often are not clinically manifest until after the agents have infected the host, replicated, and shed antigens. This is true for all viruses, but particularly those that kill the cell in which they replicate. The virus is safely tucked away, replicating. At the same time, during the incubation period, the immune system is also responding, because it was exposed to the antigen at the same time the infected mucosa was. When a host infection produces clinical manifestations, the immune system already has been in action for 4 to 5 days, plenty of time for a secondary immune response to produce multi-milligram quantities of antibody, which can rapidly deposit and incite overt nephritis.

DR. PAUL KURTIN (*Chief, Division of Pediatric Nephrology, New England Medical Center*): The use of prednisone to treat IgA nephropathy complicated by the nephrotic syndrome has met with variable success. Your data in mice that were given cyclophosphamide and in which the IgG level remained elevated suggest that this type of therapy could make the disease even worse. What can you say about immune modulators in the treatment of IgA nephropathy?

DR. EMANCIPATOR: Woo's group in Singapore [37], and Kobayashi's group in Sasamihara, Japan [152], have had success in proteinuric patients at high risk of developing end-stage renal disease and in patients who present with nephrotic-range proteinuria by treating them with prednisone, as though they had idiopathic nephrotic syndrome. Controversy exists regarding the relationship between IgA nephropathy and nephrosis: are these two processes, or is nephrosis a manifestation of IgAN? In nephrotic patients with IgA nephropathy, it seems that prednisone therapy not only reduces the proteinuria, but also increases the likelihood that patients will maintain good renal function for a longer period.

Regarding our mice given cyclophosphamide, we gave two doses, each 100 mg/kg as a single shot, and compared these mice with animals given saline injections [84]. The effects we elicited in the mice probably differ fundamentally from the effects induced with treatment of nephrotic syndrome, in which cyclophosphamide is administered at a dose of 1–2 mg/kg/day for as long as 6 months. I cannot predict the effect of sustained therapy with cyclophosphamide (1–2 mg/kg/day) on immune function in IgAN, but I agree that this approach is worth trying. Because I consider IgAN an immunologic disease, I believe that some form of immunotherapy ultimately will prove useful.

DR. NICOLAOS E. MADIAS (*Chief, Division of Nephrology, New England Medical Center*): As you know, IgA nephropathy frequently recurs in allografts, especially in grafts from living related donors, although the graft is rarely lost. Have any comparative lymphocyte studies been carried out during the pre-uremic clinical stage and also after renal transplantation?

DR. EMANCIPATOR: No, and your question prompts two other points. First, the immunologic milieu of patients with IgA nephropathy appears to be different from that in normal individuals. Save for the effects of immunosuppressive agents, that milieu shouldn't change in the presence of a renal transplant. Why, then, shouldn't IgAN recur in transplants? Second, how do dialysis and the elimination of transient or chronic uremia influence the immune dysregulation in patients with IgAN? Lymphocyte studies in patients with IgA nephropathy generally were done on patients with normal serum creatinine levels [88–97]. It would be interesting to measure lymphocyte function in the patient with an allograft. It's a very complicated system comprising the allograft itself, the immune response to the allograft, and the immunosuppressive therapy. Such studies might prove critically important. Finally, kidneys from donors with silent IgAN have been transplanted into non-IgAN hosts [153–155]. The IgAN deposits resolve, an occurrence I find fascinating. This reversal might be unexpected, but I believe that immune deposits in the glomeruli are reversible.

DR. MADIAS: Only 40% to 50% of patients with IgA nephropathy have elevated circulating levels of IgA. What differentiates these patients from the rest?

DR. EMANCIPATOR: We are in the process of looking at this issue as it relates to IL-4 or IL-5 levels. Usually subjects for lymphocyte studies have been consolidated into one group. We are stratifying patients according to disease activity and according to serum IgA levels. I am not aware of any lymphocyte function study in which patients with high levels of IgA were distinguished from those with normal serum levels of IgA, so I cannot answer your question.

DR. ANDREW S. LEVEY (*Division of Nephrology, New England Medical Center*): What have we learned from the genetic studies of families with IgA nephropathy?

DR. EMANCIPATOR: As I mentioned, many family studies suggest that some of the same defects observed in IgA nephropathy also occur in first-degree relatives. Several groups, most notably Julian and Wyatt, alone and in collaboration with Egido, have shown clear familial risk for IgAN [94, 107, 116, 117, 121, 123]. Schena and Sakai also have suggested a genetic basis for lymphocyte function abnormalities [92, 93, 156]. Julian and colleagues found changes in the nucleic acid sequences (assessed by restriction fragment length polymorphisms) in the IgG2, IgG4, and an IgG pseudogene located between the IgA1 and IgA2 genes [104]. Demaine and colleagues report increases in the frequency of homozygotes with either allele coding for the IgM switch regions in patients with IgAN [103]. Patients with a heterozygous switch region have less likelihood of developing IgA nephropathy; the relative risk is about 2.0- to 2.5-fold. Finally, Wyatt's studies clearly show that both C3 and C4 complement phenotypes, particularly C4 null alleles, are strongly related to risk for and severity of IgAN, but the

mechanism linking complement phenotypes to disease is obscure [122, 123].

DR. KURTIN: After a similar illness, Henoch-Schönlein purpura can develop in one child and isolated IgA nephropathy in a sibling. Could you speculate on what might cause one child to have a systemic manifestation of the disease and the other child to have localized disease? Also, why do the vast majority of children with Henoch-Schönlein purpura experience complete resolution of their disease?

DR. EMANCIPATOR: Let me answer the second question first. A lot of patients with Henoch-Schönlein purpura develop the disease because they have an immature immune system. If you believe the working hypothesis that I presented to you, then you have to agree that young people are at higher risk than older people for having an overly enthusiastic immune system. The difference that I see between Henoch-Schönlein purpura and IgA nephropathy is a difference of degree. That is, with more immune complexes, systemic disease is more likely. However, as the immune system matures, proper immunoregulation supervenes. I believe that in Henoch-Schönlein purpura nephritis, the disease resolves as the immune system learns to regulate itself.

Regarding your first question, an intriguing hypothesis is emerging from the data that Montinaro and Rifai have gathered from a bacterial polysaccharide-induced model of IgA nephropathy. In that model, gut lesions and purpura, or purpura and glomerular lesions as well as macrohematuria develop (personal communication). The location and severity of the lesions depend on the amount of antigen, relative amount of antibody, and strain of mice. The nature of the antigen dramatically influences the clinical expression of the disease, because different pneumococcal strains' polysaccharides behave differently. Perhaps Henoch-Schönlein purpura is an expression of an antigen-dependent process.

DR. MADIAS: Do we have any insights into the geographic distribution of IgA nephropathy?

DR. EMANCIPATOR: The incidence of IgAN differs dramatically in different countries. When expressed as a percentage of all renal biopsies, the incidence of IgAN ranges from 5% in the United States and the United Kingdom to nearly 50% in Singapore and Japan [1–7]. Although genetic and environmental factors undoubtedly influence this incidence, active urine screening and aggressive biopsy policies are important factors as well. In the nations with higher rates of diagnosis of IgAN, more IgAN patients have mild urinary abnormalities, and physicians display a higher propensity to perform renal biopsy and/or to order routine urinalysis in patients who have mild urinary abnormalities. The converse is true in countries with a low rate of renal biopsy. In countries with an apparently low incidence of IgAN, there is an inverse correlation between macrohematuria and/or proteinuria in excess of 1 g/day, whereas this correlation does not exist in countries where IgAN is frequently diagnosed [7].

DR. MADIAS: Would you speculate on the mechanisms responsible for progression of IgA nephropathy?

DR. EMANCIPATOR: We're going to need a lot more time to discover the mechanisms. Patients with episodic macrohematuria have a lot of activity in their glomeruli for a short amount of time; then they seem to return to a normal state. Patients with persistent microhematuria and/or proteinuria progress slowly but consistently over a long period. Proteinuria itself might lead to progression of the nephropathy, according to Brenner's hyperfiltration model [157]. I have an alternate view. I have a rat model that I have not presented here. This rat model is basically the mouse model that I described earlier at 6 weeks. In that system, the animals develop mixed IgG, IgA, and C3 immune deposits in the kidney, and they develop microhematuria and significant proteinuria. The proteinuria is heavy for a rat, about 20–25 mg/hour; this level is abnormal, but not near nephrotic range. We measured glomerular eicosanoid production, and we have carried out hemodynamic measurements on whole kidneys in these rats. We are collaborating with Badr on micropuncture studies. I am aware of no other model of immunologic disease in which vasoconstrictor thromboxane is increased without anti-vasoconstrictor prostaglandins being increased to a parallel if not a greater degree. Indeed, in most other models, prostaglandin E_2 levels were much higher when compared to control values than were the thromboxane levels [158, 159]. In our rat model, thromboxane is synthesized in diseased glomeruli at about 2 times its basal level. At the same time, we have seen a very slight increase in prostaglandin E_2, a rise that is not statistically significant. As one might predict, the hemodynamics are compatible with efferent arteriolar constriction, namely, renal plasma flow decreases and filtration fraction is constant. On the other hand, a thromboxane receptor antagonist plus thromboxane synthetase inhibitor can restore the renal plasma flow to basically normal levels, but under these conditions, filtration fraction is decreased at the whole-kidney level.

These observations suggest that mesangial contraction is present in this model of IgAN. In unmanipulated rats with IgAN, this contraction is offset, in terms of filtration fraction, by thromboxane-induced efferent arteriole constriction. Because contraction leads to matrix synthesis, hyperplasia, and hypertrophy in smooth muscle, it also might lead to these changes in contractile mesangial cells. Matrix synthesis would, in turn, generate sclerosis. In addition, the rich thromboxane synthesis in this model might contribute to sclerosis. Klotman has shown that thromboxane analogues stimulate matrix synthesis by mesangial cells in culture [160]. These two factors, alone or in concert, might underlie progression to glomerulosclerosis.

Reprint requests to Dr. S. Emancipator, Institute of Pathology, 2085 Adelbert Road, Cleveland, Ohio 44106, USA

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